Claims

1. Method for the specific detection of *Mycobacterium tuberculosis (M. tuberculosis)* in a biological sample in which method

   a nucleic acid amplification method is carried out using primers which are suitable for amplifying a DNA segment from the sequence shown in SEQ ID NO: 1 which sequence comprises a segment from the region of the *narGHJI* nitrate reductase operon, the DNA segment comprising position –215 in the 5' to 3' direction of reading upstream of the translation start codon GTG of the *narGHJI* nitrate reductase operon

   and in the case of which the polymorphism specific for M. tuberculosis is detected in position –215 in the 5' to 3' direction of reading upstream of the translation start codon GTG of the *narGHJI* nitrate reductase operon.

2. Method according to claim 1 characterised in that the nucleic acid amplification takes place by PCR, NASBA, SDA or LCR.

3. Method according to claim 2 characterised in that the PCR is a real time PCR.

4. Method according to claims 1 to 3 characterised in that the detection of the polymorphism specific for *M. tuberculosis* is carried out by the specific hybridisation of one or several probes.

5. Method according to claims 1 to 4 characterised in that the amplified DNA segment has a length of at last 1 and maximum 500 nucleotides.

6. Method according to claims 1 to 4 characterised in that the amplified DNA segment has a length of at least 1 and maximum 300 nucleotides.

7. Method according to claims 1 to 4 characterised in that the amplified DNA segment has a length of at least 1 and maximum 155 nucleotides.

8. Method according to claims 1 to 7 characterised in that the primer pair has at least one of the sequences having SEQ ID NO: 2 and SEQ ID NO: 3 or the complementary sequences thereof.

9. Method according to claims 1 to 8 characterised in that the detection of the polymorphism specific for *M. tuberculosis* takes place by means of at least one pair of labelled hybridisation probes, one probe being labelled at its 3' end and the other probe at its 5' end and the probes binding specifically to the amplificate in such a way that a fluorescence resonance energy transfer (FRET) is made possible.

10. Method according to claim 9 characterised in that the probe pair has the sequences having SEQ ID NO: 4 and 5 or the complementary sequences thereof and/or the sequences with SEQ ID NO: 4 and 6 or the complementary sequences thereof.

11. Method according to claims 1 to 10 characterised in that the sample is selected from the group of clinical samples consisting of saliva, bronchial lavage, gastric juice, urine, faeces, liquor, bone marrow, blood and biopsies.

12. Primer pair characterised in that it is suitable for the amplification of a DNA segment from the sequence shown in SEQ ID NO: 1 which comprises a segment from the area of the *narGHJI* nitrate reductase operon, the DNA segment comprises position –215 in the 5' to 3' direction of reading upstream of the translation start codon GTG of the *narGHJI* nitrate reductase operon.

13. Primer pair according to claim 12 characterised in that at least one primer exhibits the sequence indicated in SEQ ID NO: 2 or the complementary sequence.

14. Primer pair according to claim 12 characterised in that at least one primer exhibits the sequence indicated in SEQ ID NO: 3 or the complementary sequence.

15. Primer pair according to claim 12 characterised in that the primers exhibit the sequences indicated in SEQ ID NO: 2 and the sequences indicated in SEQ ID NO: 3 or the complementary sequences thereof.

16. Primer characterised in that it exhibits the sequence indicated in SEQ ID NO: 2 or the complementary sequence thereof.

17. Primer characterised in that it exhibits the sequence indicated in SEQ ID NO: 3 or the complementary sequence thereof.

18. Use of a primer according to claims 16 and 17 or a primer pair according to claims 13 to 16 for the specific detection of *M. tuberculosis*.

19. Hybridisation probe characterised in that it is suitable for the specific detection of the polymorphism specific for *M. tuberculosis* which is located in position –215 in the 5' to 3' direction of reading upstream of the translation start codon GTG of the *narGHJI* nitrate reductase operon.

20. Hybridisation probe characterised in that it is suitable for the specific detection of the polymorphism specific for *M. tuberculosis* which is located in position –215 in the 5' to 3' direction of reading upstream of the translation start codon GTG of the *narGHJI* nitrate reductase operon.

21. Hybridisation probe pair according to claim 20 characterised in that at least one probe exhibits the sequence indicated in SEQ ID NO: 4 or the complementary sequence thereof.

22. Hybridisation probe pair according to claim 20 characterised in that at least one probe exhibits the sequence indicated in SEQ ID NO: 5 or the complementary sequence thereof.

23. Hybridisation probe pair according to claim 20 characterised in that at least one probe exhibits the sequence indicated in SEQ ID NO: 6 or the complementary sequence thereof.

24. Hybridisation probe pair according to claim 20 characterised in that the probes exhibit the sequences indicated in SEQ ID NO: 4 and SEQ ID NO: 5 or the complementary sequences thereof.

25. Hybridisation probe pair according to claim 20 characterised in that the probes exhibit the sequences indicated in SEQ ID NO: 4 and SEQ ID NO: 6 or the complementary sequences thereof.

26. Hybridisation probe characterised in that it exhibits the sequence indicated in SEQ ID NO: 4 or the complementary sequence thereof.

27. Hybridisation probe characterised in that it exhibits the sequence indicated in SEQ ID NO: 5 or the complementary sequence thereof.

28. Hybridisation probe characterised in that it exhibits the sequence indicated in SEQ ID NO: 6 or the complementary sequence thereof.

29. Use of a hybridisation probe according to claims 26 to 28 or a hybridisation probe pair according to claims 21 to 25 for the specific detection of *M. tuberculosis*.

30. Kit for carrying out a method according to claims 1 to 11.

31. Kit according to claim 30 which
comprises at least one primer pair which is suitable for the amplification of a DNA segment from the sequence shown in SEQ ID NO: 1 which comprises a segment from the area of the *narGHJI* nitrate reductase operon, the DNA segment comprising position –215 in the 5' to 3' direction of reading upstream of the translation start codon GTG of the *narGHJI* nitrate reductase operon and/or

comprises at least one hybridisation probe or a hybridisation probe pair which is suitable for the specific detection of the polymorphism specific for *M. tuberculosis* which is located in position –215 in the 5' to 3' direction of reading upstream of the translation start codon GTG of the *narGHJI* nitrate reductase operon.

32. Kit according to claim 31 characterised in that the primer pair is the primer pair according to claim 15.

33. Kit according to claim 31 characterised in that the hybridisation probe pair is the probe pair according to claim 24 and/or the probe pair according to claim 25.

34. Kit according to claim 33 characterised in that the primer pair is the primer pair according to claim 15.

35. Kit according to claims 30 to 34 characterised in that it comprises further reagents and/or auxiliary agents necessary or useful for carrying out a nucleic acid amplification and/or detection reaction.

36. Method for the specific detection of *mycobacterium tuberculosis (M. tuberculosis)* in clinical material comprising the steps

a) extraction of microbial DNA from clinical material,

b) amplification of at least one DNA fragment of the promoter region of the *narGHJI* nitrate reductase operon of mycobacteria containing at least one DNA polymorphism specific for *M. tuberculosis* from the extracted DNA,

c) detection of the specific hybridisation of the amplified DNA fragment with at least one hybridisation probe which comprises the nucleotide sequence selected from the group consisting of SEQ ID NO: 5, the complementary sequence to SEQ ID NO: 5, SEQ ID NO: 6, and the complementary sequence to SEQ ID NO: 6,

the specific detection of *M. tuberculosis* vis-à-vis *M. bovis, M. bovis* BCG, *M. africanum* and *M. microti* taking place by way of melting curve analysis.

37. Method according to claim 36, the DNA polymorphism being located in position – 215 of the promoter region in the 5' to 3' direction of reading upstream of the translation start codon GTG of the *narGHJI* nitrate reductase operon.

38. Method according to claim 37 the amplification taking place in step b) by means of a primer pair comprising the nucleotide sequences SEQ ID NO: 2/SEQ ID NO: 3.

39. Method according to one of claims 36 to 38, the specific hybridisation taking place in step c) with at least one pair of labelled hybridisation probes comprising the nucleotide sequences SEQ ID NO: 4/SEQ ID NO: 5 or the pair of complementary sequences thereof and/or the nucleotide sequences SEQ ID NO: 4/SEQ ID NO: 6 or the pair of complementary sequences thereof.

40. Method according to one of the preceding claims, the multiplication of the DNA fragments by polymerase chain reaction (PCR) being carried out preferably as real time PCR, preferably be means of the LightCycler™ system.

41. Method according to one of the preceding claims, the steps of specific hybridisation taking place during or after the amplification of the DNA fragments.

42. Method according to one of the preceding claims, the specific hybridisation and its detection taking place in real time PCR, preferably in the LightCycler™ system.

43. Method according to one of the preceding claims, the detection of the specific hybridisation being carried out by fluorescence detection and the labelled hybridisation probe pairs being formed as fluorescence resonance energy transfer (FRET) pair.

44. Method according to one of the preceding claims, the clinical material being selected from the group of clinical samples consisting of saliva, bronchial lavage, gastric juice, urine, faeces, liquor, bone marrow, blood and biopsies.

45. Oligonucleotide primer pair for the amplification of a DNA fragment of the *M. tuberculosis*-specific promoter region of the *narGHJI* nitrate reductase gene comprising the nucleotide sequences SEQ ID NO: 2/SEQ ID NO: 3.

46. Oligonucleotide which hybridises specifically with a *M. tuberculosis*-specific promoter region of the *narGHJI* nitrate reductase operon comprising the nucleotide sequence SEQ ID NO: 5 or the complementary sequence thereof.

47. Oligonucleotide which hybridises specifically with a *M. tuberculosis*-specific promoter region of the *narGHJI* nitrate reductase operon comprising the nucleotide sequence SEQ ID NO: 6 or the complementary sequence thereof.

48. Oligonucleotide pair comprising the nucleotide sequences SEQ ID NO: 4/SEQ ID NO: 5 or the pair of complementary sequences thereof.

49. Oligonucleotide pair comprising the nucleotide sequences SEQ ID NO: 4/SEQ ID NO: 6 or the pair of complementary sequences thereof.

50. Kit for the specific detection of *Mycobacterium tuberculosis* comprising

a) at least one primer pair comprising the nucleotide sequences SEQ ID NO: 2/SEQ ID NO: 3 and

b) at least one hybridisation probe pair comprising the nucleotide sequences SEQ ID NO: 4/SEQ ID NO: 5 or the pair of complementary sequences thereof and/or at least one hybridisation probe pair comprising the nucleotide sequences SEQ ID NO: 4/SEQ ID NO: 6 or the pair of complementary sequences thereof.

51. Use of a *M. tuberculosis*-specific DNA polymorphism in the promoter region of the *narGHJI* nitrate reductase operon of mycobacteria containing the nucleic acid sequence represented in SEQ ID NO: 6 or the complementary sequence thereof, for the specific detection of an infection with *M. tuberculosis*.

Figure 1: Comparison of the *narGHJI* promoter of *M. tuberculosis* (TB) *M. bovis* (Bovis) and *M. bovis* BCG (BCG)

Figure 2: Abscissa: temperature (°C)
Ordinate: fluorescence (dl/dt)

Figure 4: Nitrate reductase test: *M. tuberculosis* Wild type and mutants, *M. bovis* and *M. bovis* BCG as a comparison.

Figure 5: 215 SNP with 1500 bp flanked sequences
1500 bases before and behind the –215 SNP ("T") in the promoter of the narGHJI operon

Sequence listing

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Method and kit for the specific detection of M. tuberculosis